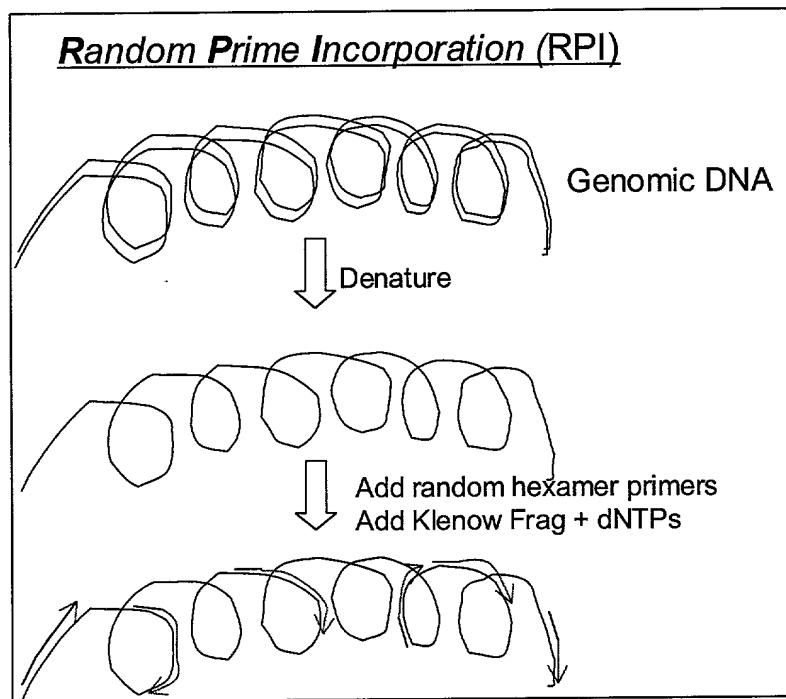


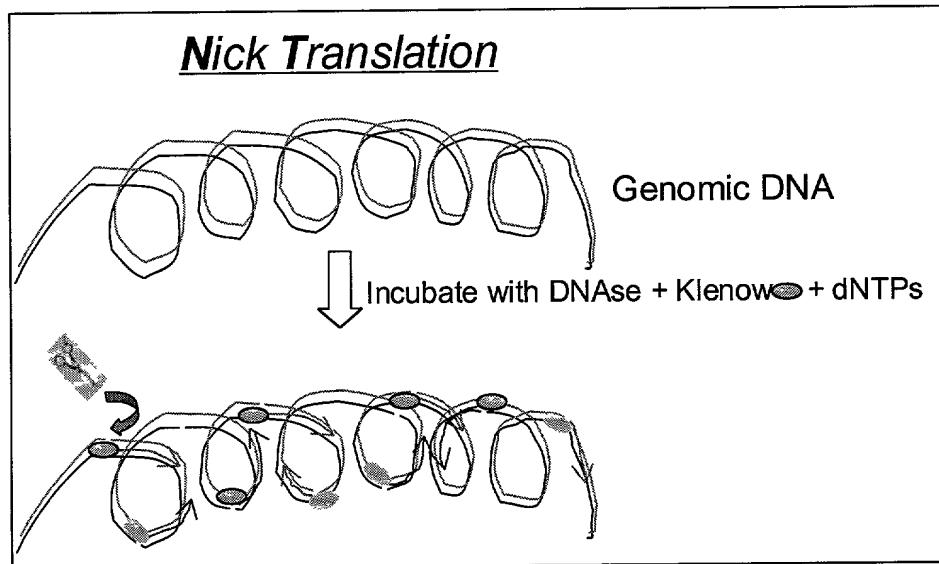
1/8

Figure 1



2/8

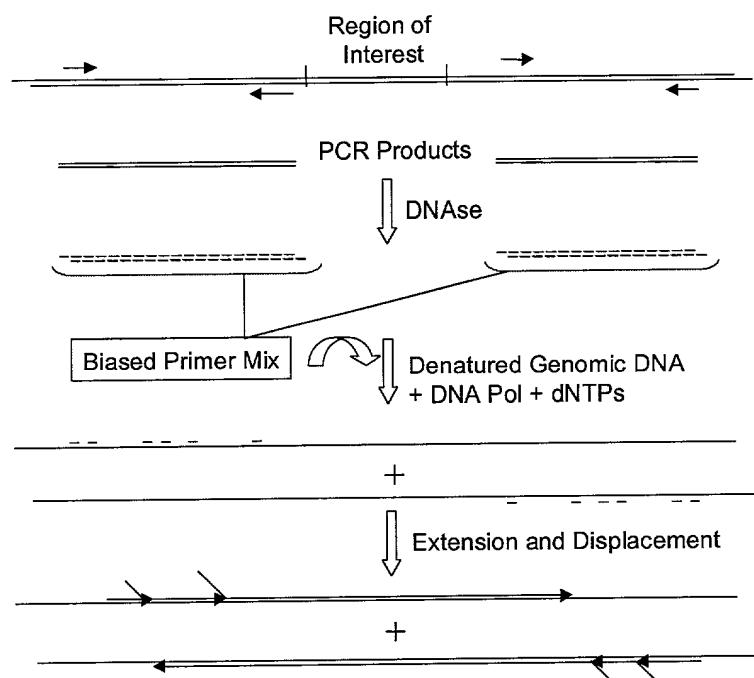
Figure 2



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Figure 3

*Biased Primer Extension
Generated by DNase-Digested PCR Products*



4/8

Figure 4

Gene-Specific Primer Extension using DNA Polymerase

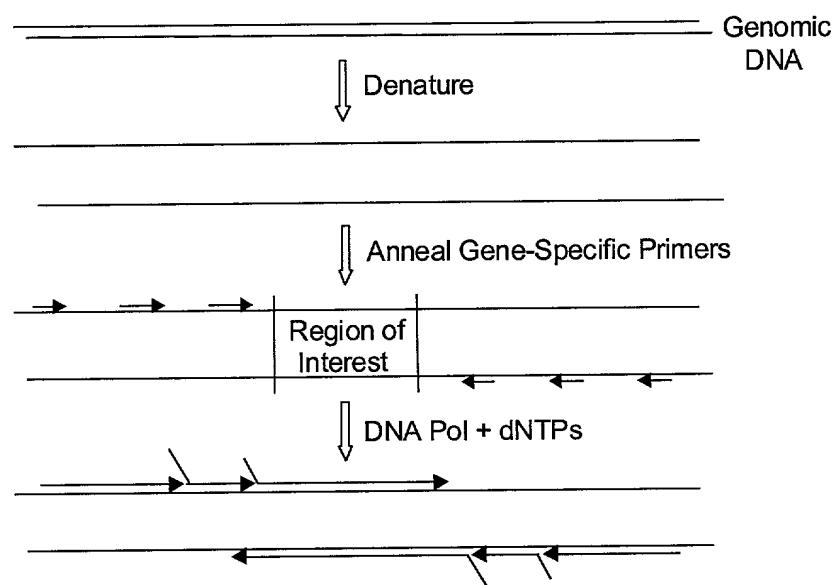


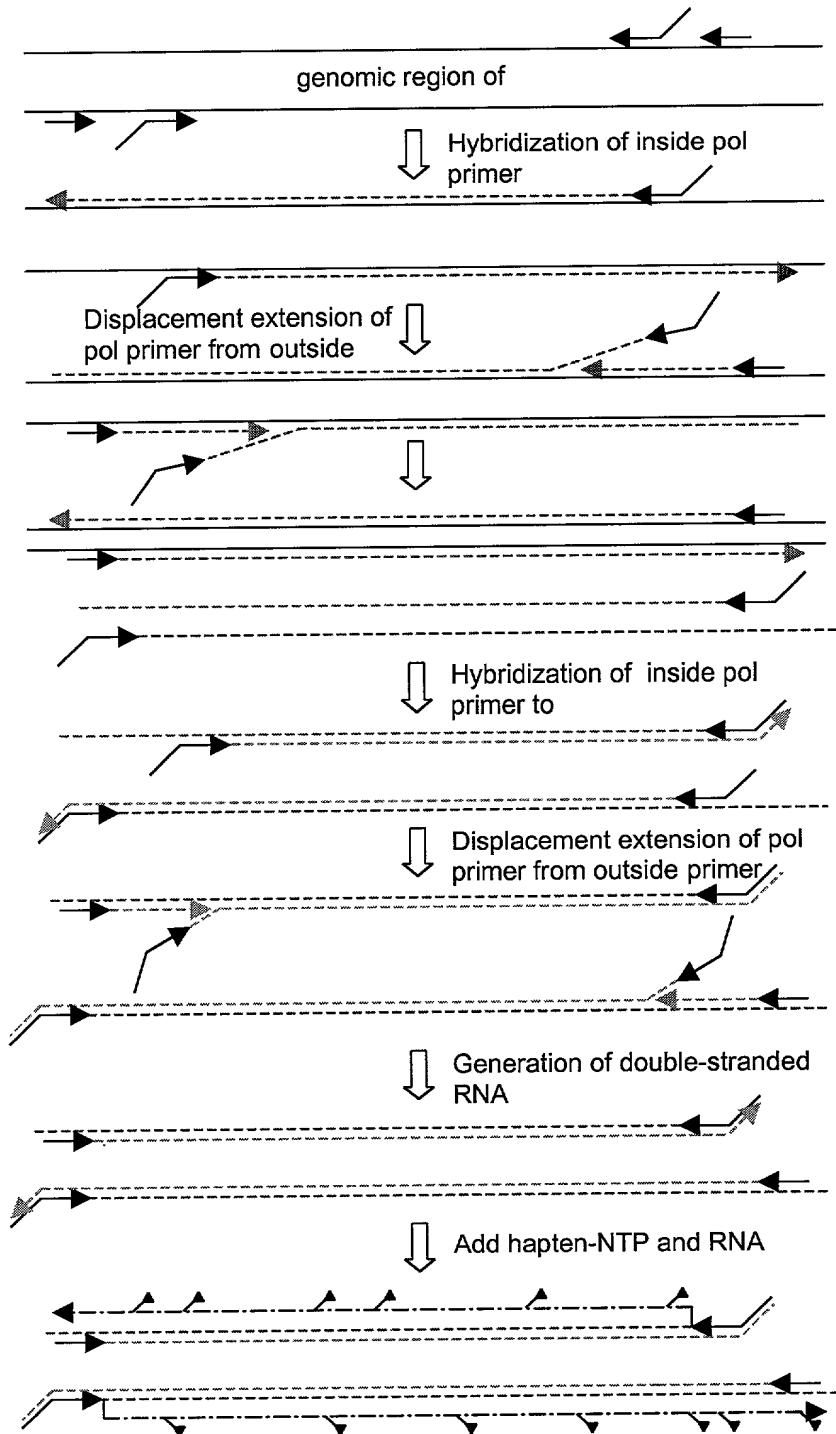
Figure 5**Extension Displacement Transcription Incorporation (EDTI)**

Figure 6

1. 'A' Allele, CYP2D6*3, A₂₆₃₇ deletion, Frameshift resulting in zero enzyme activity

2637

5'- G C T A A C T G A G C A C A G G A T G A C C -3' NH2 CYPwt(+)A2624,22mer,54%GC, Tm=63-64C
 5'- G C T A A C T G A G C A C A G G A T G A C C (A)30-3' NH2 CYPwt(+)A2624(A)30-3'NH2
 5'- C T A A C T G A G C A C A G G A T G A C C (A)30-3' NH2 CYPwt(+)A2625(A)30-3'NH2
 5'- C T A A C T G A G C A C A G G A T G A C (A)30-3' NH2 CYPwt(+)A2625b(A)30-3'NH2
 5'- G C T A A C T G A G C A C - G G A T G A C C -3' NH2 CYPmut(+)A2624,21mer,57%GC, Tm=61-63C
 5'- G C T A A C T G A G C A C - G G A T G A C C (A)30-3' NH2 CYPmut(+)A2624(A)30-3'NH2
 5'- C T A A C T G A G C A C - G G A T G A C C (A)30-3' NH2 CYPmut(+)A2625(A)30-3'NH2
 5'- C T A A C T G A G C A C - G G A T G A C (A)30-3' NH2 CYPmut(+)A2625b(A)30-3'NH2
 -2612 5'- G C T G G A T G A G C T G C T A A C T G A G C A C - G G A T G A C C T G G G A C C C A G C C C A G C C -3' Wild Type (+)
 5'- G C T G G A T G A G C T G C T A A C T G A G C A C - G G A T G A C C T G G G A C C C A G C C C A G C C -3' Mut (+)

2. 'B' Allele, CYP2D6*4, G₁₉₃₄A, Splicing defect resulting in zero enzyme activity

A. wt Probe - CYPwt(-)B1922 (C/A to mut at base 5) & CYPmut(+)B1922 (A/C to mut at base 13)

1934

NH2 3'- G A G G G T G G G G G T C C T G C -5' CYPwt(-)B1922,17mer,76%GC, Tm=66C
 5'- C T C C C A C C C C C A G G A C G -3' NH2 CYPwt(+)B1922- Target
 5'- C T C C C A C C C C C A A G A C G -3' NH2 CYPmut(+)B1922,17mer,71%GC, Tm=58-60C
 -1909 NH2 3'- G A G G G T G G G G G T T C T G C -5' CYPmut(-)B1922- Target
 5'- C C C T T A C C C G C A T C T C C C A C C C C C A G G A C G C C C C T T C G C C C C A A C G G T C T -3' Wild Type (+)
 5'- C C C T T A C C C G C A T C T C C C A C C C C C A A G A C G C C C C T T C G C C C C A A C G G T C T -3' Mut (+)

B. CYPwt(-)B1930 (C/A to mut at base 13) and CYPmut(+)B1930 (A/C to wt at base 5)

NH2 3'- G G G T C C T G C G G G G A A A G -5' CYPwt(-)B1930,17mer,71%GC, Tm=56C
 NH2 3'- (A)30 G G G T C C T G C G G G G A A A G -5' CYPwt(-)B1930(A)30-3'NH2
 5'- C C C A A G A C G C C C C T T C -3' NH2 CYPmut(+)B1930,17mer,65%GC, Tm=54C
 -1909 5'- C C C A A G A C G C C C C T T C (A)30-3' NH2 CYPmut(+)B1930(A)30-3'NH2
 5'- C C C T T A C C C G C A T C T C C C A C C C C C A G G A C G C C C C T T C G C C C C A A C G G T C T -3' Wild Type (+)
 5'- C C C T T A C C C G C A T C T C C C A C C C C C A A G A C G C C C C T T C G C C C C A A C G G T C T -3' Mut (+)

3. 'C' Allele, CYP2D6*9, G₂₇₀₂-A₂₇₀₄ deletion, decreased enzyme activity

-2702

5'- G C A G A G A T G G A G A A G G T G A G A G -3' NH2 CYPwt(+)C2691,22mer,55%GC, Tm=60C
 5'- G C A G A G A T G G A G A A G G T G A G A G (A)30-3' NH2 CYPwt(+)C2691(A)30-3'NH2
 5'- C A G A G A T G G A G A A G G T G A G A G (A)30-3' NH2 CYPwt(+)C2692(A)30-3'NH2
 5'- G C A G A G A T G G A - - - G G T G A G A G T G -3' NH2 CYPmut(+)C2691,21mer,57%GC, Tm=60C
 5'- G C A G A G A T G G A - - - G G T G A G A G T G (A)30-3' NH2 CYPmut(+)C2691(A)30-3'NH2
 -2676 5'- C A G A G A T G G A - - - G G T G A G A G T G (A)30-3' NH2 CYPmut(+)C2692(A)30-3'NH2
 5'- T G A C T C C G G A A G G A C C G T C T A C C T C T T C C A C T C T C A C C G A C G G T G C C A C -5' Wild Type (-)
 5'- T G A C T C C G G A A G G A C C G T C T A C C T C T T C C A C T C T C A C C G A C G G T G C C A C -5' Mut (-)

4. 'E' Allele, CYP2D6*7, A₃₀₂₃C, H₃₂₄P amino acid change results in zero enzyme activity

A. wt Probe - CYPwt(-)E3009 (T/C to mut at base 5) & CYPmut(+)E3009 (C/A to wt at base 15)

3023

NH2 3'- C G A G T A C T A G G A T G T A G G C -5' CYPwt(-)E3009,19mer,53%GC,Pred Tm=57
 NH2 3'- (A)30 C G A G T A C T A G G A T G T A G G C -5' CYPwt(-)E3009(A)30-3'NH2
 5'- G C T C A T G A T C C T A C C T C C G -3' NH2 CYPmut(+)E3009,19mer,58%GC,Pred Tm=59C
 -2998 5'- G C T C A T G A T C C T A C C T C C G (A)30-3' NH2 CYPmut(+)E3009(A)30-3'NH2
 5'- T G G G G C T C C T G C T C A T G A T C C T A C A T C C G G A T G T G C A G C G T G A G C C C A T C -3' Wild Type (+)
 5'- T G G G G C T C C T G C T C A T G A T C C T A C C T C C G G A T G T G C A G C G T G A G C C C A T C -3' Mut (+)
 -3038-Intron Start

B. CYPwt(-)E3018 (T/C to mut at base 14) and CYPmut(+)E3018 (C/T to wt at base 6)

NH2 3'- G G A T G T A G G C C T A C A C G T C -5' CYPwt(-)E3018,19mer,58%GC, Tm=60
 5'- C C T A C A T C C C G G A T G T G C A G -3' CYPwt(+)E3018- Target
 5'- C C T A C C T C C C G G A T G T G C A G -3' NH2 CYPmut(+)E3018,19mer, 63%GC, Tm=62C
 -2998 5'- G G A T G G A G G C C T A C A C G T C -5' CYPmut(-)E3018- Target
 5'- T G G G G C T C C T G C T C A T G A T C C T A C A T C C C G G A T G T G C A G C G T G A G C C C A T C -3' Wild Type (+)
 5'- T G G G G C T C C T G C T C A T G A T C C T A C C T C C C G G A T G T G C A G C G T G A G C C C A T C -3' Mut (+)
 -3038-Intron Start

5. 'G' Allele, CYP2D6*8, G₁₈₄₆T, Stop codon, zero enzyme activity

1846

5'- C A C T C C G G T G G G T G A T G G (A)30-3' NH2 CYPwt(+)G1840(A)30-3'NH2,18mer,67%GC, Tm=60
 NH2 3'- (A)30 G T G A G G C C A C C C A C T A C C -5' CYPwt(-)G1840(A)30-3'NH2
 5'- C A C T C C T G T G G G T G A T G G (A)30-3' NH2 CYPmut(+)G1840(A)30-3'NH2,18mer,61%GC, Tm=57
 5'- G T G C C G C C T T C G C C A C T C C | G G T G G G T G A T G G G C A G A A G G G C A C A A A G C G G G -3'
 5'- G T G C C G C C T T C G C C A C T C C | T G T G G G T G A T G G G C A G A A G G G C A C A A A G C G G G -3'
 Exon 3 end- -1846

6. 'T' Allele, CYP2D6*6, T1795 deletion, Frameshift resulting in zero enzyme activity

5'- G C T G G A G C A G T G G G T G A C -3' NH2	CYPwt(+)T1785,18mer,67%GC, Tm=59-61C
5'- G C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)T1785(A)30-3'NH2
5'- C T G G A G C A G T G G G T G A C (A)30-3' NH2	CYPwt(+)T1786(A)30-3'NH2
5'- G C T G G A G C A G - G G G T G A C -3' NH2	CYPmut(+)T1785,17mer,71%GC, Tm=58-60C
5'- G C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)T1785(A)30-3'NH2
5'- C T G G A G C A G - G G G T G A C (A)30-3' NH2	CYPmut(+)T1786(A)30-3'NH2
5'- G G G C A A G A A G T C G C T G G A G C A G T G G G T G A C C G A G G A G G G C C G C C T G C C T -3'	Wild Type (+)
5'- G G G C A A G A A G T C G C T G G A G C A G - G G G T G A C C G A G G A G G G C C G C C T G C C T -3'	Mut (+)

7. 2D6/2D7/2D8 Controls - The 2D6/7/8 probes were designed in the 1600 region of the 2D6 gene. The purpose of the designs was to find region somewhere between the PCR primers were it would be easy to discriminate between 2D6 and its two pseudogenes, 2D7 and 2D8. The purpose of the designs is to demonstrate that the PCR amplicon is from the 2D6 gene, not one of the pseudogenes.

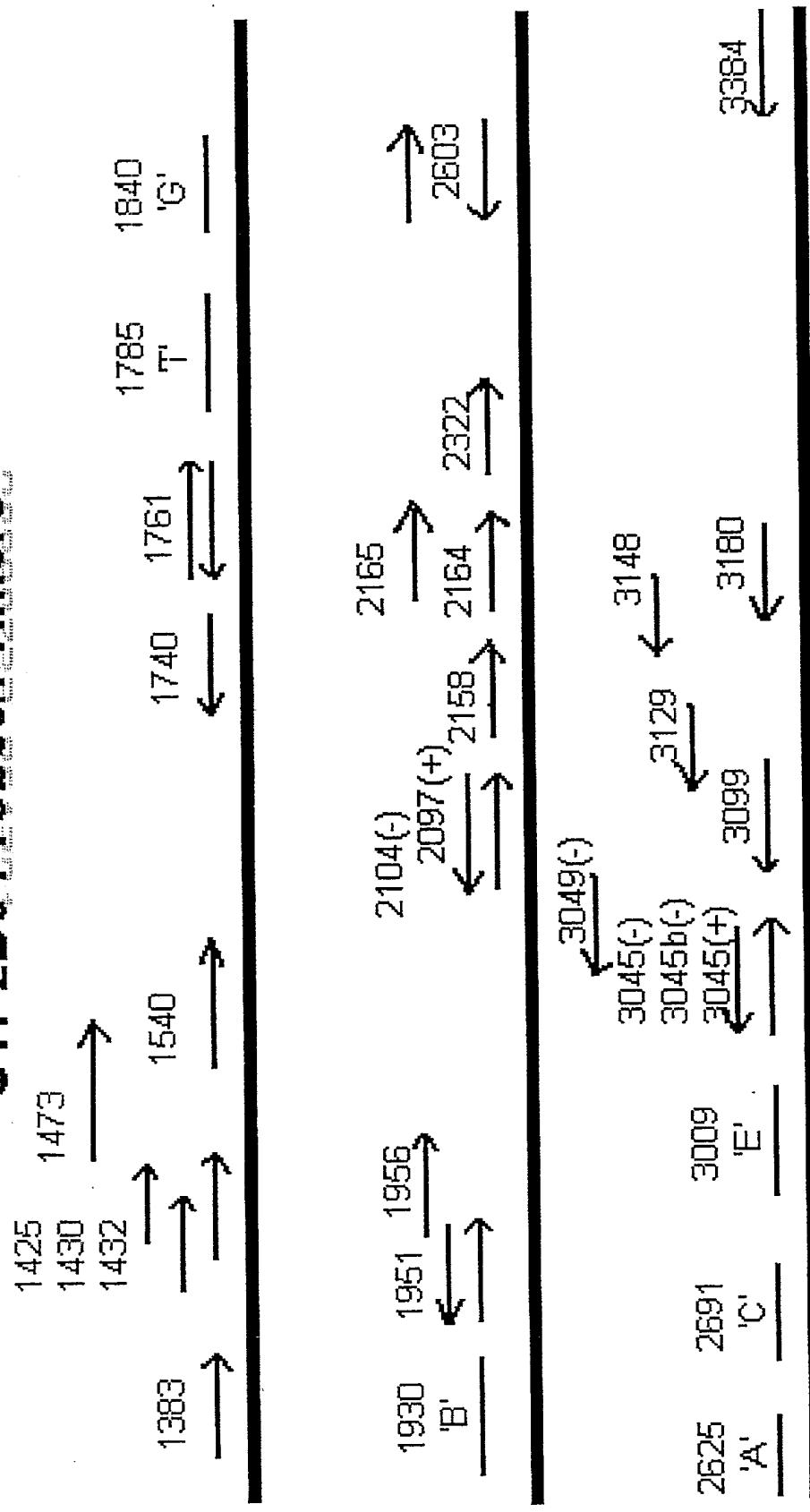
5'- G A C C A G G G G A G C - A T A G G (A)30-3' NH2	CYP2D6wt(+)1607(A)30-3'NH2
5'- G A C C T T G T G G A G C G C C A G (A)30-3' NH2	CYP2D7wt(+)1607(A)30-3'NH2
5'- G A C C A G G A A A A G C - A C A G G (A)30-3' NH2	CYP2D8wt(+)1607(A)30-3'NH2
1603- 5'- G A C C A G G A A A A G C - A C A G G G (A)30-3' NH2	CYP2D8wt(+)1607b(A)30-3'NH2
5'- G G G A G A C C A G G G G A G C - A T A G G G T T G G A G T G G G T G G T -3'	2D6 (+)
5'- G G G A G A C C T T G T G G A G C G C C A G G G T T G G A G T G G G T G G C -3'	2D7 (+)
5'- G G G A G A C C A G G A A A A G C - A C A G G G T T G G A G T G G G C G G C -3'	2D8 (+)

8. Pos/Neg Control probes- These probes were designed as true positive and negative control probes. They consist of the same semi-random sequence, with the positive control probe having a 5' Biotin.

5' Biotin- A T C A T T C C A A T C A T C A T C A T C (A)25-3' NH2	CYP(+)ran(A)25-5'Biotin,3'NH2
5'- A T C A T T C C A A T C A T C A T C A T C (A)25-3' NH2	CYP(+)ran(A)25-3'NH2

Figure 7

CYP2D6 Primers



CYPwt(+)1383, CYPwt(+)2097, CYPwt(-)2104, and CYPwt(-)3180 are published primer sequences.

1. Chen et al., Clinical Pharmacology and Therapeutics, Vol 60, 5:522-34.
2. Heim M, Meyer UA. Lancet 1990; 336:529-32

CYPwt(+)1540 and CYPwt(-)3099 are primers obtained from Intek, referred to as MP3 and MP4 respectively.